PA TENT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 10 July 2001 (10.07.01)	
International application No. PCT/US00/25512	**Applicant's or agent's file reference** VET 1 WO
International filing date (day/month/year) 18 September 2000 (18.09.00)	**Priority date** (day/month/year) 17 September 1999 (17.09.99)
Applicant TRUCKSIS, Michele	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

12 April 2001 (12.04.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Nestor Santesso
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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PCT

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US 60/154,322 (CIP)
Filed on 17 September 1999 (17.09.1999)

(71) Applicants *(for all designated States except US)*: **UNIVERSITY OF MARYLAND, BALTIMORE** [US/US]; 520 West Lombard Avenue, Baltimore, MD 21201 (US). **UNITED STATES GOVERNMENT, as represented by DEPARTMENT OF VETERANS AFFAIRS** [US/US]; 810 Vermont Avenue, N.W., Washington, DC 20420 (US).

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:
— *with international search report*

(88) Date of publication of the international search report:
22 November 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/19993 A3

(54) Title: VIRULENCE GENES OF *M. MARINUM* AND *M. TUBERCULOSIS*

(57) Abstract: Methods for identifying, isolating and mutagenizing virulence genes of mycobacteria, *e.g., M. marinum* and *M. tuberculosis*, are described. Also described are isolated virulence genes and fragments of them, isolated gene products and fragments of them, avirulent bacteria in which one or more virulence genes are mutagenized, attenuated vaccines containing such mutant bacteria, and methods to elicit an immune response in a host, using such mutant bacteria.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/25512

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/13 C07K14/35 A61K38/00 A61K39/00 A61P31/04
C12N9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, EMBL, BIOSIS, EMBASE, MEDLINE, CAB Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A. TALAAT ET AL.: "Use of signature-tagged mutagenesis to identify Mycobacterium marinum genes required for in vivo survival in the goldfish model of Mycobacterial infection." 99TH GENERAL MEETINF OF THE AMERICAL SOCIETY FOR MICROBIOLOGY, ABSTRACTS IN MICROBIAL PATHOGENESIS/GENERAL MEDICAL MICROBIOLOGY, vol. 99, no. 29/B/D, Abstract B/D-15, 30 May 1999 (1999-05-30) - 3 June 1999 (1999-06-03), pages 31-32, XP000978661 Chicago, Illinois, USA	1-5,50
Y	abstract	28-42, 51-76
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 March 2001	11 06 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hix, R

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/25512

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLE S T ET AL: "Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence" NATURE,MACMILLAN JOURNALS LTD. LONDON,GB, vol. 393, no. 6685, 11 June 1998 (1998-06-11), pages 537-544, XP002154587 ISSN: 0028-0836 the whole document	28,31, 35,51, 54,58
X	WO 99 09186 A (PORTNOI DENIS ;GUIGUENO AGNES (FR); LIM ENG MONG (FR); GICQUEL BRI) 25 February 1999 (1999-02-25) the whole document	28,51
X	DATABASE EMBL [Online] Accession number: AI592454, 26 April 1999 (1999-04-26) M. MARRA ET AL.: "The WashU-NCI mouse EST Project 1999" XP002161752 sequence data abstract	29,52
Y	TALAAT A.M. ET AL: "Goldfish, Carassius auratus, a novel animal model for the study of Mycobacterium marinum pathogenesis." INFECTION AND IMMUNITY, (1998) 66/6 (2938-2942)., XP000979750 the whole document	1-5, 28-42, 50-76
Y	A. TALAAT ET AL.: "Goldfish, Carassius auratus a fish model for mycobacterial disease." 96TH GENERAL MEETING OF THE AMERICAL SOCIETY FOR MICROBIOLOGY, vol. 96, no. U-177, 19 - 23 May 1996, page 132 XP000978662 New Orleans, Louisiane, USA abstract	1-5, 28-42, 50-76
P,X	TALAAT A M ET AL: "Transformation and transposition of the genome of Mycobacterium marinum." AMERICAN JOURNAL OF VETERINARY RESEARCH, (2000 FEB) 61 (2) 125-8., XP000981305 the whole document	1-5, 28-42, 50-76

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/25512

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	L. RAMAKRISHNAN ET AL.: "Granuloma-specific expression of Mycobacterium virulence proteins from the Glycine-rich PE-PGRS family." SCIENCE, vol. 288, 26 May 2000 (2000-05-26), pages 1436-1439, XP000979218 the whole document	1-5, 28-42, 50-76
A	RINDI L. ET AL: "Search for genes potentially involved in Mycobacterium tuberculosis virulence by mRNA differential display." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (29 APR 1999) 258/1 (94-101)., XP000979851 the whole document	
A	A. KIRAN KINGER ET AL.: "Identification and cloning of genes differentially expressed in the virulent strain of Mycobacterium tuberculosis." GENE, vol. 131, 1993, pages 113-117, XP000978684 the whole document	
A	K. SRIVASTAVA ET AL.: "Immunogenic behaviour of Mycobacterium marinum (SATO) in mice." THE INDIAN JOURNAL OF MEDICAL RESEARCH, vol. 84, 1986, pages 485-491, XP000979839 the whole document	
A	T. TONJUM ET AL.: "Differentiation of Mycobacterium ulcerans, M. marinum and M. haemophilum: Mapping of thier relationships to M. tuberculosis by fatty actid profile analysis, DNA-DNA Hybridization and 16S rRNA gene sequence analysis." JOURNAL OF CLINICAL MICROBIOLOGY, vol. 36, no. 4, April 1998 (1998-04), pages 918-925, XP000979751 cited in the application the whole document	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/25512

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 46 and 47 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-5, 28-42, 46, 50-76

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-5, 28-42, 46, 50-76

Method for identifying a virulence gene of M. marinum, avirulent M. marinum, isolated nucleic acid comprising oligonucleotide of SEQ ID NO: 4, 6, 8, 11, 13, 21, 23, 25, 27, 29, 31, 39, 41 and nucleic acids which is complementary to or which can hybridize under conditions of high stringency to a portion of said nucleic acid identified by said SEQ ID NOs, pharmaceutical composition comprising avirulent M. marinum bacterium, attenuated M. marinum vaccine comprising said avirulent bacterium, method for isolating a mutagenized M. marinum bacterium which exhibits reduced virulence in a host.

2. Claims: 6-27, 43-45, 47

Method for identifying a virulence gene of M. tuberculosis, method for generating avirulent M. tuberculosis bacterium, avirulent M. tuberculosis comprising one or more mutated genes according to claims 9-27, pharmaceutical composition comprising avirulent M. tuberculosis bacterium, attenuated M. tuberculosis vaccine comprising said avirulent bacterium.

3. Claim : 48

An isolated polyketide made by the M. marinum polyketide synthase gene.

4. Claim : 49

An isolated polyketide made by the M. tuberculosis polyketide synthase gene.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/25512

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9909186 A	25-02-1999	FR 2767336 A	19-02-1999
		FR 2767337 A	19-02-1999
		AU 9076598 A	08-03-1999
		EP 1003888 A	31-05-2000

M

PATENT COOPERATION TREATY

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RECD 11 FEB 2002 PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
VET 1 WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/25512	18/09/2000	17/09/1999
International Patent Classification (IPC) or national classification and IPC C12N15/13		
Applicant UNIVERSITY OF MARYLAND		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12/04/2001	11.02.2002
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Montero Lopez, B Telephone No. +31 70 340 3739

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/25512

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-60 as originally filed

Claims, No.:

1-76 as originally filed

Drawings, sheets:

1/16-16/16 as originally filed

Sequence listing part of the description, pages:

1-17, filed with the letter of 1-11-2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 6-27, 43-45, 47-49 and claims 46 and 47 with respect to industrial applicability.

because:

   ☒ the said international application, or the said claims Nos. 46 and 47 with respect to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 6-27, 43-45, 47-49.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	30, 32-34, 36-42, 46, 53, 55-57, 59-76
	No:	Claims	1-5, 28, 29, 31, 35, 50- 52, 54, 58
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-5, 28-42, 50-76
Industrial applicability (IA)	Yes:	Claims	1-5, 28-42, 50-76
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 46 and 47 relate to subject-matter considered by this authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: A. TALAAT ET AL.: 'Use of signature-tagged mutagenesis to identify Mycobacterium marinum genes required for in vivo survival in the goldfish model of Mycobacterial infection.' 99TH GENERAL MEETING OF THE AMERICAL SOCIETY FOR MICROBIOLOGY, ABSTRACTS IN MICROBIAL PATHOGENESIS/GENERAL MEDICAL MICROBIOLOGY, vol. 99, no. 29/B/D, Abstract B/D-15, 30 May 1999 (1999-05-30) - 3 June 1999 (1999-06-03), pages 31-32, XP000978661 Chicago, Illinois, USA

D2: COLE S T ET AL: 'Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence' NATURE,MACMILLAN JOURNALS LTD. LONDON,GB, vol. 393, no. 6685, 11 June 1998 (1998-06-11), pages 537-544, XP002154587 ISSN: 0028-0836

D3: WO 99 09186 A (PORTNOI DENIS ;GUIGUENO AGNES (FR); LIM ENG MONG (FR); GICQUEL BRI) 25 February 1999 (1999-02-25)

D4: DATABASE EMBL [Online] Accession number: AI592454, 26 April 1999 (1999-04-26) M. MARRA ET AL.: 'The WashU-NCI mouse EST Project 1999' XP002161752

D5: TALAAT A.M. ET AL: 'Goldfish, Carassius auratus, a novel animal model for the study of Mycobacterium marinum pathogenesis.' INFECTION AND

IMMUNITY, (1998) 66/6 (2938-2942)., XP000979750

1. D1 discloses the use of an IS1096-derived transposon and signature tagged mutagenesis to identify genes of *Mycobacterium marinum* required for in vivo survival in a goldfish model, in order to screen for virulence factors. Transposition of IS1096 in the *M. marinum* genome was random and the first screening resulted in the identification of 13 attenuated mutants. The abstract concludes that the "application of STM to screen for virulence genes of *M. marinum* may allow the identification of the first virulence gene of this organism."

1.1. The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1 to 5 and 50 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

2. D2 discloses the complete genome sequence of *M. tuberculosis*, where the sequence Accession number MTV043 has a 95.799% identity in 169 nucleotides with SEQ ID NO: 4 of the present application, a 78.571% identity in 244 nucleotides with SEQ ID NO: 11 and a 73.169% identity in 388 nucleotides with SEQ ID NO: 25.

2.1. Consequently in the light of the disclosure of D2, the subject-matter of claims 28, 31, 35, 51, 54 and 58 is not novel according to Article 33(2) PCT.

3. D3 also discloses nucleic acid sequences from Mycobacteria, including sequence Accession number X34160 which has a 85.799% identity in 169 nucleotides with SEQ ID NO: 4 of the present application.

3.1. Consequently in the light of the disclosure of D3, the subject-matter of claims 28 and 51 is not novel according to Article 33(2) PCT.

4. D4 discloses a sequence Accession number AI592454 which has a 96.957% identity in 230 nucleotides with SEQ ID NO: 6 of the present application.

4.1. Consequently in the light of the disclosure of D4 the subject-matter of claims 29 and 52 is not novel according to Article 33(2) PCT.

5. The subject-matter of claims 30, 32-34, 36-42, 46, 53, 55-57 and 59-76 has not been disclosed in the state of the art and, therefore, these claims are novel according to Article 33(2) PCT.

6. Document D5 is considered to represent the most relevant state of the art. D5 discloses the animal model for studying *Mycobacterium marinum* using goldfish, where the paper concludes, page 2942, that the authors "plan to use this model to screen for potential virulence mutants of *M. marinum*." The subject-matter of claims 30, 32 to 34, 36 to 40, 53, 55 to 57 and 59 to 76 involves *M. marinum* virulence genes.

6.1. The problem to be solved by the subject matter of claims 30, 32 to 34, 36 to 40, 53, 55 to 57 and 59 to 76 may therefore be regarded as the provision of *M. marinum* virulence genes where the solution is provided by the nucleic acid sequences of claims 30, 32 to 34, 36 to 40, 53, 55 to 57 and 59 to 76. This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) as the person skilled in the art is already directed towards the screening for potential virulence mutants of *M. marinum* through the use of the goldfish model of D5. Considering the above problem the person skilled in the art would apply the teachings of D1, using the goldfish model of D5 and as a matter or routine identify the virulence genes of *M. marinum*. The present application does therefore not satisfy the criterion set forth in Article 33(3) PCT as the subject-matter of claims 30, 32 to 34, 36 to 40, 53, 55 to 57 and 59 to 76 does not involve an inventive step (Rule 65(1)(2) PCT).

6.2. The preparation of a pharmaceutical composition or an attenuated *M. marinum* vaccine comprising an avirulent *M. marinum* bacterium, as known from D1, combined with a pharmaceutically acceptable carrier, is a matter of routine not considered to involve an inventive step on behalf of the person skilled in the art. The subject-matter of claims 41 and 42 is therefore also considered not to involve an inventive step according to Article 33(3) PCT.

7. For the assessment of the present claims 46 and 47 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to

the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII
Certain defects in the international application

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in document D1 is not mentioned in the description.

Re Item VIII
Certain observations on the international application

1. The application does not meet the requirements of Article 6 PCT because claims are not clear for the following reasons:

1.1. Claims 28 to 40 and 51 to 76 include fragments or variants of the claim nucleic acid, however the fragments or variants are not defined in terms of any technical features. Due to the fact that said fragments or variants are so vaguely defined, known fragments are also encompassed by such a general definition, according to Articles 6 and 33(2) PCT. Such broad claims to the polynucleotide fragments lack a functional limitation in order to clearly and unambiguously distinguish the fragments claimed in the present application from any generally known fragments.

1.2. Furthermore, use of the term "consisting essentially of..." used in claims 51 to 63 also renders the scope of the claims unclear and open to interpretation.

1.3. It appears from the description as a whole that the virulence genes identified by the SEQ ID NOs are an essential technical feature of the present invention. This essential technical feature is however not present in independent claims 1 and 50. Said claims therefore lack clarity according to Art. 6 PCT taken in combination with Rule 6.3 (b) PCT (see also PCT Preliminary Examination

Guidelines III.4.3).

1.4. Claim 50 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. Through the use of the term "in a manner effective to produce..." the claim attempts to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result are however missing.

10/129348

Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
T 3173-Cs/Bi		
International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/EP00/10673	30 October 2000 (30.10.00)	02 November 1999 (02.11.99)

International Patent Classification (IPC) or national classification and IPC
A61F 2/28

Applicant
TUTOGEN MEDICAL GMBH

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☒ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05 April 2001 (05.04.01)	04 February 2002 (04.02.2002)
Name and mailing address of the IPEA/EP	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP00/10673

I. Basis of the report

1. This report has been drawn on the basis of (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

☒ the international application as originally filed.

☒ the description, pages 3-8, as originally filed,
pages _____, filed with the demand,
pages 1,1a,2, filed with the letter of 07 January 2002 (07.01.2002).
pages _____, filed with the letter of _____.

☒ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-4, filed with the letter of 07 January 2002 (07.01.2002).
Nos. _____, filed with the letter of _____.

☒ the drawings, sheets/fig 1/1, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: II.3

The validity of the priority claimed has not been checked.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-4	YES
	Claims		NO
Inventive step (IS)	Claims	1-3	YES
	Claims	4	NO
Industrial applicability (IA)	Claims	1-4	YES
	Claims		NO

2. Citations and explanations

1. This report makes reference to the following documents:

D1: EP-A-0 834 294
D2: DE-A-195 43 110
D3: US-A-4 436 684.

2. Document D3, which is considered to be the prior art closest to the subject matter of Claim 1, discloses (cf. column 2, lines 3-50; column 15, lines 32-59) a method of fabricating a bone implant, in which method there is made of the patient's defective body bone a computer tomogram, with the aid of which an artificial model is made of the defective body bone, a settable impression compound being poured into the region of the bone defect on the model, and the impression compound being set to form a moulded piece.

The subject matter of Claim 1 differs from the method defined in D3, firstly in that the fabricated bone implant is suitable for the purpose of fitting an artificial socket into a defective bone; secondly

in that the impression compound is moulded for this purpose in such a way that it forms a seat for an artificial socket; and, thirdly, in that scanning of the set moulded piece enables there to be a created on a cutting machine a replica of the moulded piece which is cut from a piece of preserved cancellous bone.

The subject matter of Claim 1 is therefore novel (PCT Article 33(2)).

The problem addressed by the present invention can therefore be seen as being to provide a method of fabricating a bone implant for the purpose of fitting an artificial socket into a defective bone, whereby the planning and operation of a bone defect can be simplified, the implant thus obtained being made from a material that is suitable for the treatment of bone defects and can be inserted into the bone defect to give an accurate fit.

The solution to this problem as proposed in Claim 1 of the present application involves an inventive step (PCT Article 33(3)) for the following reasons.

- The use of a machine similar to that in method step d) of Claim 1 is described in D3 (see column 3, lines 10-17). In D3, however, the machine is used either to fabricate the bone implant from a piece of synthetic material (cf. column 12, lines 38-48; column 5, lines 26-33) directly from the computer tomogram data relating to the bone defect, or to make a mould which is used to cast the bone implant (cf. column 15, lines 32-59).

- The use of a cutting machine to fabricate a bone implant from a piece of preserved cancellous bone is neither known nor obvious from any of the available prior art documents.

- The fabrication, using an artificial model of the body bone, of a moulded piece from a settable impression compound in order to create a replica of the moulded piece from a further material on a cutting machine by scanning is neither known nor obvious from the available prior art.

3. Claims 2 and 3 are dependent on Claim 1 and therefore also satisfy the PCT novelty and inventive step requirements.

4. The subject matter of independent Claim 4 does not involve an inventive step and does not therefore satisfy the criterion of PCT Article 33(3). The reasons are as follows.

Claim 4 relates to a bone implant which is made out of preserved natural cancellous bone and which can be fabricated by a method according to any one of Claims 1 to 3 of the present application.

Document D1, which is considered to be the prior art closest to Claim 4, discloses (cf. column 5, lines 13-40; and Figure 6) a bone implant to support the fitting of an artificial socket into a defective bone, which implant can be made by a method according to any one of Claims 1 to 3.

The subject matter of Claim 4 differs from the implant described in D1 in that it is made from

preserved natural cancellous bone.

The problem addressed by the present invention can therefore be seen as being to provide a bone implant from a material that is suitable for the treatment of bone defects, that retains the biomechanical properties of the lost bone substance, and that can be reconstructed.

The subject matter of Claim 4 concerns only a new use of the material "preserved natural cancellous bone" which is known from D2. At the same time, however, the only properties of said material that are used are those likewise already known from D2, column 3, line 48 to column 4, line 12. The subject matter of Claim 4 does not therefore involve an inventive step.

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

1. Contrary to PCT Rule 5.1(a)(ii), the description does not cite D1, D2 and D3 or indicate the relevant prior art disclosed therein.

2. The description is not consistent with the claims (PCT Rule 5.1(a)(iii)).